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REPORTS

STAPHYLOCOCCAL TOXIC EPIDERMAL NECROLYSIS: SPECIES AND TISSUE SUSCEPTIBILITY AND RESISTANCE

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The staphylococcal exfoliatin, which is responsible for the "scalded skin syndrome" in man, cleaves the epidermis directly beneath the stratum granulosum. Its activity in vivo is paralleled in organ cultures, providing a rapid and convenient assay. The cutaneous responses of several mammalian and nonmammalian species were examined both in vivo and in vitro. Human and murine skin, as well as that of hamsters and monkeys exfoliated, while all other species tested (rat, rabbit, guinea pig, dog, frog, and chicken) were refractory. Results were identical in vivo and in vitro. Susceptibility and resistance are inherent, presumably genetic, attributes of the epidermis, since neither dermal elements nor circulating factors interfered with or influenced sensitivity to staphylococcal exfoliatin. Besides possessing species specificity, this exfoliatin is also tissue specific, failing to cleave all mouse nonkeratinizing epithelia tested, while the reactions of some extracutaneous keratinizing epithelia were equivocal. The species and tissue specificity of the staphylococcal scalded-skin syndrome may be attributable to either keratinocyte receptors specific for exfoliatin or the presence of specific, as yet undefined, substances in the intercellular space.

The staphylococcal "scalded-skin syndrome," a distinctive form of toxic epidermal necrolysis [1], occurs primarily in childhood [2–6]. In 1970, Melish and Glasgow [7] induced a similar clinical disorder in neonatal mice by injecting them with certain bacteriophage Group 2 staphylococcal isolates obtained from patients undergoing exfoliation. The exfoliatin (exfoliative toxin, epidermolytic toxin, epidermolysin) elaborated by these organisms was subsequently isolated, characterized, and demonstrated to cause a similar syndrome in newborn mice [8–10]. Currently, both adult mouse and human skin have proved sensitive to exfoliatin [11], and some of the factors which constitute a favorable milieu for the development of the scalded-skin syndrome in adults have been identified [12]. Our recently described in vitro model system [11] permitted comparison of epidermal sensitivity of adults and newborns, as well as comparison of the relative effects in glabrous vs hairy sites in the same individual.

In the studies reported here, we used the in vitro system: (1) to assess the sensitivity of several mammalian and two nonmammalian species to staphylococcal exfoliatin; (2) to compare the in vivo and in vitro susceptibility of a number of species to exfoliatin, and thereby (3) to devise a useful method for screening additional tissues and species for susceptibility (thus obviating the necessity for in vivo testing); and (4) to examine the sources of species-susceptibility and resistance.

MATERIALS AND METHODS

Animals and human material. Wild-type and Swiss albino mice were utilized for all experiments, including the in vivo bioassay of exfoliatin fractions (see below). Other species and strains tested were wild-type rats, wild-type guinea pigs, New Zealand white rabbits, Syrian hamsters, mongrel dogs, frogs (*Rana pipiens*), white Leghorn chickens, and free-ranging rhesus monkeys.

Several different epithelia from neonatal mice undergoing exfoliation were studied for evidence of an exfoliatin effect. At times when the skin first became spontaneously wrinkled, the esophagus, palate, stomach, bladder, and ureter were removed from matched exfoliatin-treated and sham-injected control animals, and

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prepared for electron microscopy. In addition, we obtained freshly dissected newborn mouse skin and esophagus, adult mouse skin, cervix, vagina, endometrium, adrenal glands, and epididymis, as well as monkey buccal mucosa for the incubation studies detailed below. Viable human skin from several sites was obtained at surgery and incubated as described.

Organisms, isolation of exfoliatin, and bioassay. In all experiments, preparations of exfoliatin from one of two strains of staphylococci were used. In an earlier paper [12], we reported the activity and effects of strain 1—a Group 2, phage type 71 organism. Strain 2, recently isolated from an adult patient with the scalded-skin syndrome [13], is classified as a Group 2, type 3C/71 pathogen. Exfoliatin-rich fractions were harvested from culture supernatants and partially purified [12], then refrigerated in solution or lyophilized until used. In all matched studies, we employed exfoliatin from the same batch. Batch potency was determined by bioassay, i.e., injection of serial dilutions into randomly selected neonatal mice from a number of separate litters [7]. Control preparation supernatant fractions were prepared from a non-Group-2, coagulase-positive staphylococcal strain which elaborated alpha, beta, and delta toxins but no exfoliatin [12], and partially purified as exfoliatin-containing supernatants.

Organ culture system and assay for in vitro activity. Full-thickness pieces of skin from all species tested were scraped free of excess subcutaneous tissue and floated dermis side down in plastic culture dishes containing Eagle's minimal essential medium in an atmosphere of moist air (37°C) as previously described [11,14]. Other epithelia were floated mucosal side downward, or completely immersed. Exfoliatin activity was assessed by the following methods [11] after 2, 4, 6, and 8 hr: (1) peeling, i.e., the ease with which superficial layers could be detached with fine forceps from underlying epidermis; (2) exfoliation on replicate smears obtained by "teasing" tissue samples in a drop of saline (Fig. 1); (3) histologic evidence of cleavage (samples were fixed in formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin); and (4) ultrastructural examination.

Preparation of epidermal sheets. Sheets of epidermis were obtained by two different techniques; using either method resulted in comparable findings: (1) Full-thickness skin of newborn mice and rats was incubated in 0.2 M ethylenediaminetetraacetic acid (EDTA) in 0.85% phosphate-buffered saline (PBS) at 37°C for 90 min, and the epidermis was separated from the dermis with fine forceps; or (2) "suction blisters" were produced by subjecting the back skin of neonatal mice and rats to a negative pressure of 150 to 200 mm Hg for 10 to 20 min by the use of a Dermovac suction blister device [15] (Fig. 2). Clean separation at the dermoepidermal junction was histologically proved for both types of epidermal sheets (Figs. 3,4). The effect of exfoliatin was then assessed in smears and by histologic techniques after incubations in exfoliatin-containing or control medium in the same fashion as full-thickness skin.

Electron microscopy. For ultrastructural examination, specimens were first immersed for 1 hr in a 1:1 dilution of freshly prepared Karnovsky's fixative [16] and subsequently carefully minced. By employing this combination of prefixation before mincing, we were able to minimize artifactual or additive cleavage. After a total of 5 hr of fixation at room temperature, the specimens were washed 3 times in 0.1 M cacodylate buffer (pH 7.4), postfixed in unbuffered 3% osmium tetroxide for 1.5 hr at 4°C, dehydrated, and embedded in Epon [17]. We stained thin sections with uranyl acetate and lead citrate [18] and examined them in either a Zeiss EM 9S or Siemens 1A electron microscope.

RESULTS

In Vivo Susceptibility of Various Mammalian Species to Exfoliatin

Sensitivity to isolated exfoliatin is not unique to neonatal and adult humans and mice. Whereas newborn and adult rats, guinea pigs, rabbits, and dogs of the strains tested (Tab. I) failed to respond to intradermal injections of exfoliatin (even in massive doses), both newborn and adult hamsters proved sensitive to exfoliatin. Histologic observations of injected, nontraumatized sites revealed subgranular cleavage indistinguishable from that found in human and murine skin (Figs. 5–8), but no cleavage was detectable microscopically in rat, guinea pig, rabbit, and dog skin. The process, generalized in neonates (regardless of route of administration), remained localized to skin overlying inoculated sites in adult hamsters. Thus the pattern of susceptibility exactly paralleled that in humans and mice. Neonatal hamsters, however, responded more slowly than mice (Tab. II), even with the dose of exfoliatin adjusted for differences in individual animal weight. Of these species, newborn mice, rats, and hamsters are hairless, while rabbits, guinea pigs, and dogs have hair at birth.

In an additional experiment, 9 3-day-old hamsters were inoculated with 10^9 strain 1 staphylococci. After 12 hr, 5 out of 9 manifested toxic epidermal necrolysis; after 24 hr, the skin of all animals wrinkled spontaneously. A positive Nikolsky's sign was first demonstrable at 10 hr.

Finally, two nonmammalian species, the frog (*Rana pipiens*) and the white Leghorn chicken, were injected with exfoliatin. No response was detectable, either grossly or histologically.

In Vitro Response to Exfoliatin

Quantitative aspects. The in vitro system was used to: (1) assess species-susceptibility quantitatively; (2) compare topographical individual susceptibility in the same animal(s); and (3) determine the influence of age upon susceptibility. As already reported, we have found that hairy and glabrous skin from the same species was equally sensitive in vitro [11]. In this study we found the sensitivity of adult, juvenile, and newborn mouse skin comparable to each other and to that of adult human skin (Tab. III).

Correlation of species sensitivity in vivo and in vitro. In those species tested both in vivo and in vitro, susceptibility (and resistance) to exfoliatin correlated precisely (Tab. I). Subsequently we tested monkeys by means of the in vitro system alone—monkey skin from various sites was discovered to be sensitive to the toxin (Tab. I, Fig. 6).

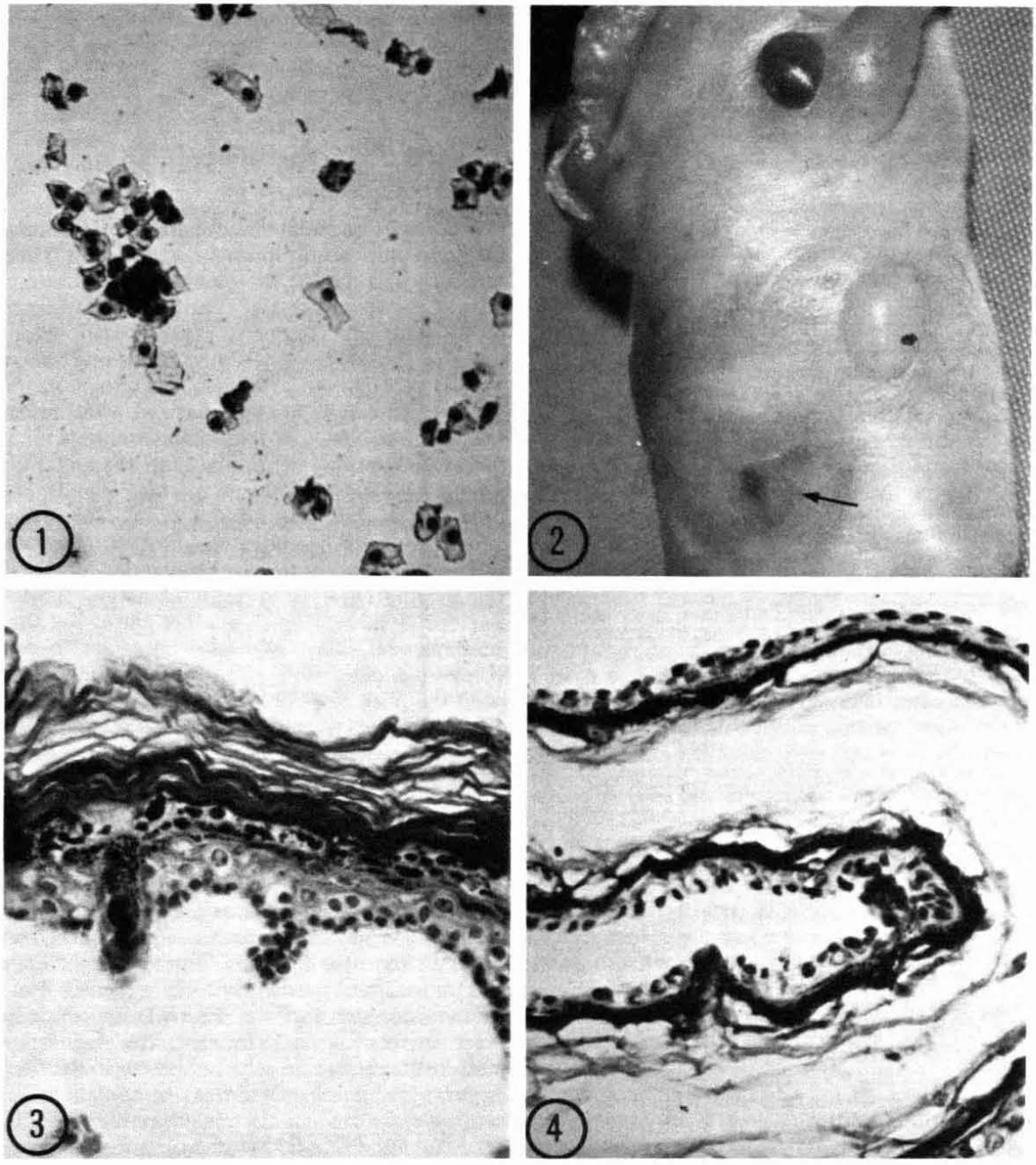


FIG. 1. Human full-thickness skin, exposed to exfoliatin for 2 hr in vitro. This Giemsa-stained smear of a "teased" specimen reveals predominantly individual, flattened squames from the upper epidermis (× 1,000).

FIG. 2. Induction of suction blisters in the neonatal rat. The noteworthy elements here are one clear bulla, a hemorrhagic one, and a denuded area (*arrow*) where the specimen has already been removed for incubation (see text).

FIG. 3. Neonatal mouse epidermal sheet. After 90-min incubation with EDTA (see text), the epidermis separates cleanly from the dermis as an intact sheet. Note the lack of spongiosis and acantholysis (× 1,000).

FIG. 4. Suction-induced epidermal sheet from a neonatal mouse. Clean epidermal–dermal separation has occurred; but, in this case, the specimen is derived from a slightly older animal. Note that total epidermal thickness is reduced (cf Fig. 2) (× 1,000).

Extracutaneous Tissue Response

Several keratinizing and nonkeratinizing epithelia, from both neonatal mice undergoing necrolysis in vivo and specimens from adult humans or mice experiencing the analogous process in vitro (Tab. IV), were examined for evidence of intercellular cleavage. We observed a possible extracutaneous effect in neonatal mouse esophagus (Figs. 9–12), human (Fig. 8) and murine genital mucosa, and monkey oral mucosa. Frank intraepithelial acantholysis, however, was not as apparent as in the epidermis. Instead, enhanced desquamation of cornified cells was noted (Fig. 11, *inset*). As in epidermis, cleaved cells seemed uninjured ultrastructurally, the predominant effect being extracellular (Figs. 11, 12). Other keratinizing epithelia, e.g., cervical and palatal, were evidently less susceptible, while nonkeratinizing epithelia were uniformly refractory (Tab. IV).

Source(s) of Species Resistance

In certain experiments, we sought source(s) of species resistance (Tab. V), and considered the following questions:

1. Does the skin of resistant species elaborate a substance which inactivates exfoliatin? In these studies, 7 to 10 1-cm² pieces of full-thickness, neonatal mouse, rat, and guinea-pig skin were incubated in separate Petri dishes containing exfoliatin (final concentration: 5 mg/ml) in 3 ml of medium. At 2 hr, the newborn mouse skin was easily peeled and disclosed mid-epidermal cleavage in histologic preparations and cell separation in smears, while guinea-pig and rat skin failed to respond. Culture fluid taken at termination of incubations with mouse, rat, and guinea-pig skin was serially diluted and injected into neonatal mice, where wrinkling resulted from concentrations as low as 0.5 mg/ml in each case. In a second

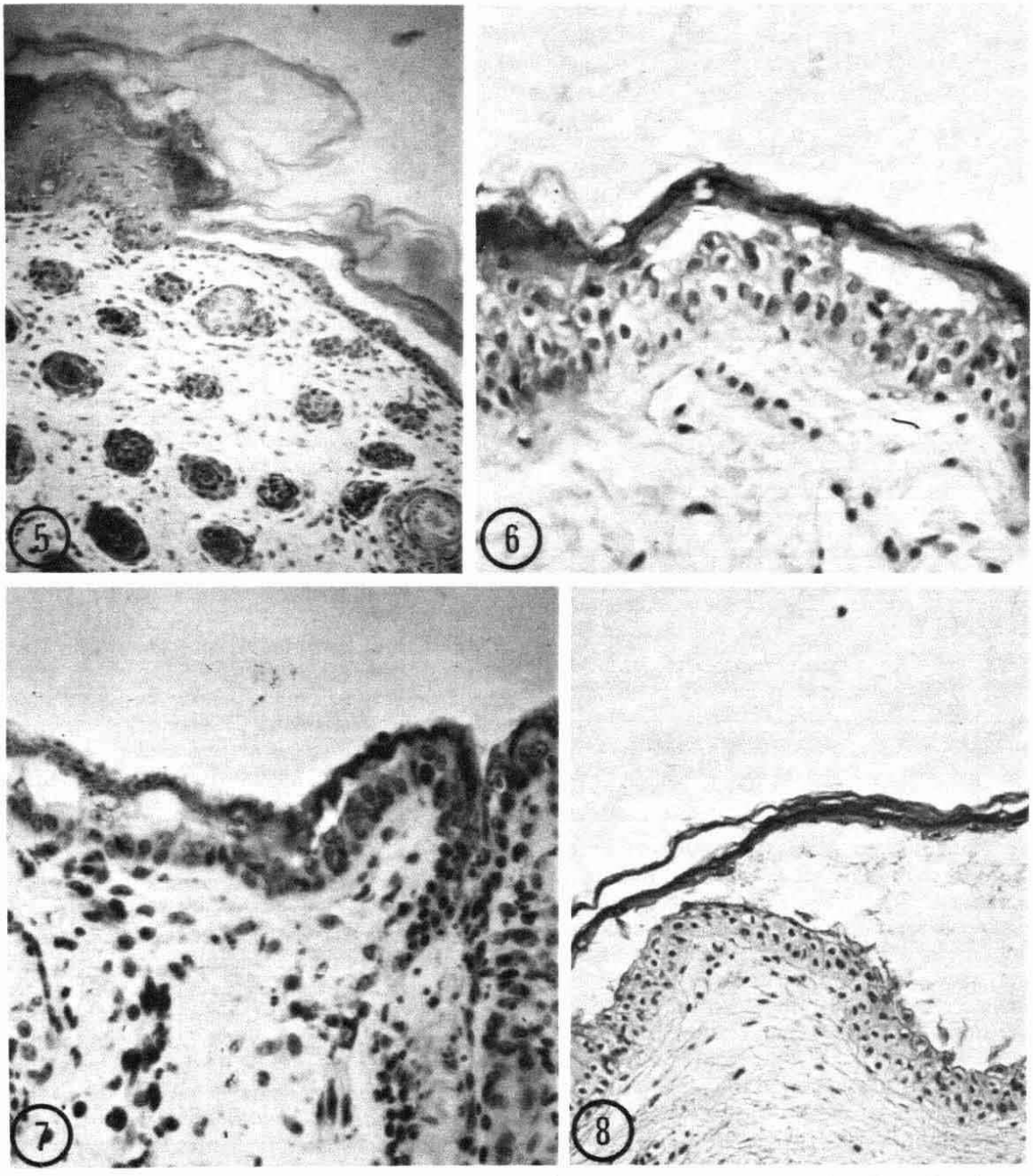


FIG. 5. Neonatal hamster skin, exposed to exfoliatin for 2 hr in vitro. A prominent mid-epidermal, subgranular cleavage plane is depicted (× 350).

FIG. 6. Monkey skin, exposed to exfoliatin for 2 hr in vitro. Microvesicles form immediately beneath the granular layer (× 1,000).

FIG. 7. Ten-day-old mouse skin, exposed to exfoliatin for 2 hr in vitro. Cleavage occurs directly under the stratum granulosum, without regard to the presence of adjacent hair structures (× 1,000).

FIG. 8. Full-thickness human vulvar mucous membrane, exposed to exfoliatin for 2 hr in vitro, exhibits a large upper epidermal bulla. (The granular layer is not particularly conspicuous in this tissue.) (× 650)

TABLE I. *Cutaneous susceptibility or resistance of several species to exfoliatin*

	Species	Age group tested	Sites injected in vivo	Source of skin specimen for incubations
Susceptible	Human	Juvenile	Not tested	Dorsal forearm
		Adult	Forearm, thigh	Multiple regions
	Mouse	Newborn	All routes	Multiple regions
		Adult	All sites	Multiple regions
	Hamster	Newborn	All sites	Multiple regions
		Adult	Ear	Not tested
	Monkey	Adult	Not tested	Eyelid, abdomen
Resistant[a]	Rat	Newborn	All sites	Multiple regions
		Adult	Flank, ear	Ear
	Guinea pig	Newborn	All sites	Flank, ear
		Adult	Flank, ear	Ear
	Rabbit	Newborn	All sites	Flank, ear
		Adult	Ear	Ear
	Dog	Adult	Ear	Ear
	Frog	Adult	Flank	Flank, thigh
	Chicken	Juvenile	Flank	Flank

[a] A negative in vivo response in neonates and adults is based upon the failure of the animal to react to 100 times the exfoliatin dose/gm body weight of minimum required to induce wrinkling in newborn mice (0.01–0.05 mg).

TABLE II. *Comparative responses of newborn mice and newborn hamsters to exfoliatin administered in vivo*[a]

Time after injection (*hr*)	Toxic epidermal necrolysis	
	Newborn mouse (*% responding*)	Newborn hamster (*% responding*)
1	25–75	0
2	100	25
4	100	50
6	100	75

[a] At least 6 animals from 3 separate litters were tested at each time. The dose of toxin was adjusted to 1 mg/gm body weight.

TABLE III. *Adult human and mouse skin of various ages: quantitative comparison of response to exfoliatin in vitro*[a]

Tissue	Exfoliatin concentration	Time exposed to exfoliatin		
		1 Hr	2 Hr	4 Hr
Human (adult)[b]				
	3 mg/ml[b]	+	+	+
	3×10^{-1}	+/–	+	+
	3×10^{-2}	–	+/–	+
	3×10^{-3}	–	–	–
Mouse (adult)				
	3 mg/ml	–	+/–	+
	3×10^{-1}	–	–	+
	3×10^{-2}	–	–	–
	3×10^{-3}	–	–	–
Mouse (juvenile)				
	3 mg/ml	–	+	+
	3×10^{-1}	–	+/–	+
	3×10^{-2}	–	–	+/–
	3×10^{-3}	–	–	–
Mouse (newborn)				
	1.5 mg/ml	–	+	+
	1.5×10^{-1}	–	–	+
	1.5×10^{-2}	–	–	+/–
	1.5×10^{-3}	–	–	–

[a] Response was assessed by the following manifestations: peeling, exfoliative cytology, cleavage in histologic sections, and, in some cases, ultrastructural modifications.

[b] The same batch of exfoliatin was used in determining all dose–response curves.

set of experiments, pieces of newborn rat and mouse skin were exposed to the exfoliatin in the same culture dish. Once again, mouse skin was cleaved, whereas rat skin remained unaltered. Moreover, the activity of postincubation medium from these mixed cultures was undiminished when injected into murine neonates. Observations gleaned from these experiments suggest that the in vitro resistance of rat and guinea-pig skin cannot be attributed to the elaboration of an exfoliatin-neutralizing substance into the culture fluid.

2. Does the plasma of resistant species contain an agent which inactivates exfoliatin? In two separate experiments we diluted exfoliatin (10 mg/ml) 1:1 with fresh heparinized plasma obtained from 6 or 7 adult mice and 3 adult rats. After 5 to 10 minutes of mixing at room temperature, serial dilutions were injected into groups of 3

TABLE IV. *Susceptibility of various human, monkey, and murine epithelia to exfoliatin*[a, b]

Species	Epithelium	In vivo	In vitro
Mouse			
	Glabrous skin (N, A)	Positive (1, 2, 3, 4)	Positive (1, 2, 3, 4)
	Hairy skin (A)	Positive (1, 3, 4)	Positive (1, 2, 3)
	Esophagus (N)	Positive (3, 4)	Positive (3, 4)
	Vagina (A)	Not tested	Positive (1, 2, 3)
	Cervix (A)	Not tested	Negative (1, 2, 3)
	Endometrium (A)	Not tested	Negative (1, 2, 3)
	Bladder (N)	Negative (3)	Not tested
	Ureter (N)	Negative (3)	Not tested
	Stomach (N)	Negative (3)	Not tested
	Adrenal gland (N)	Not tested	Negative (3, 4)
	Epididymis (N)	Not tested	Negative (3, 4)
Human			
	Glabrous skin (A)	Positive (1, 2, 3, 4)	Positive (1, 2, 3, 4)
	Hairy skin (A)	Positive (1, 3)	Positive (1, 3, 4)
	Vulvar mucosa (A)	Not tested	Positive (1, 3)
Monkey			
	Glabrous skin (A)	Not tested	Positive (1, 2, 3)
	Mucous membrane (A) (oral mucosa)	Not tested	Positive (1, 2, 3)

[a] N = neonatal; A = adult.
[b] Assessed by: 1 = peeling; 2 = exfoliative cytology; 3 = histology; 4 = electron microscopy.

neonatal mice as above. Incubation with rat plasma produced no diminution of potency. Similarly, full-thickness newborn mouse skin exposed to exfoliatin (10 mg/ml) in vitro with equal parts medium and either rat or mouse plasma responded identically well. These findings suggest that rats possess no circulating factor which rapidly inactivates exfoliatin.

3. Does dermis confer resistance? Both suction-induced and EDTA-separated epidermal sheets from neonatal mice and newborn rats were incubated with exfoliatin (5 mg/ml) for 2 hr. We prepared cytologic and histologic material from duplicate specimens and read these "blind" for evidence of acantholysis. While neonatal mouse preparations revealed numerous individual, flattened, acantholytic cells (Fig. 1), smears of similarly treated rat epidermis exhibited only shredded clumps and large cellular aggregates, but few or no individual cells. Histologically, mouse epidermal sheets demonstrated cleavage at the characteristic level (Fig. 13, cf Figs. 3, 4). Rat epidermis, however, appeared unchanged.

In another related series of experiments, newborn mouse and rat full-thickness skin was first split with EDTA and subsequently mutual recombinants (i.e., overlying mouse epidermis onto rat dermis, and rat epidermis onto mouse dermis) were formulated. Exfoliatin-containing or control medium (5 mg/ml) was then added to the various recombinants. After 2 hr of exposure, recombinants of mouse epidermis plus rat dermis again revealed cleavage similar to that of control preparations of full-thickness mouse skin, but the rat epidermis plus mouse dermal recombinants remained unmodified.

TABLE V. *In vitro comparison of newborn mouse and newborn rat skin sensitivity to exfoliatin*[a]

Sensitive	Resistant
Mouse skin (full-thickness)	Rat skin (full-thickness)
Mouse skin in mixed mouse and rat organ-cultures	Rat skin in mixed mouse and rat organ-cultures
Mouse skin in rat plasma	Rat skin in mouse plasma
Mouse epidermal sheets	Rat epidermal sheets
Mouse epidermis recombined with rat dermis	Rat epidermis recombined with mouse dermis

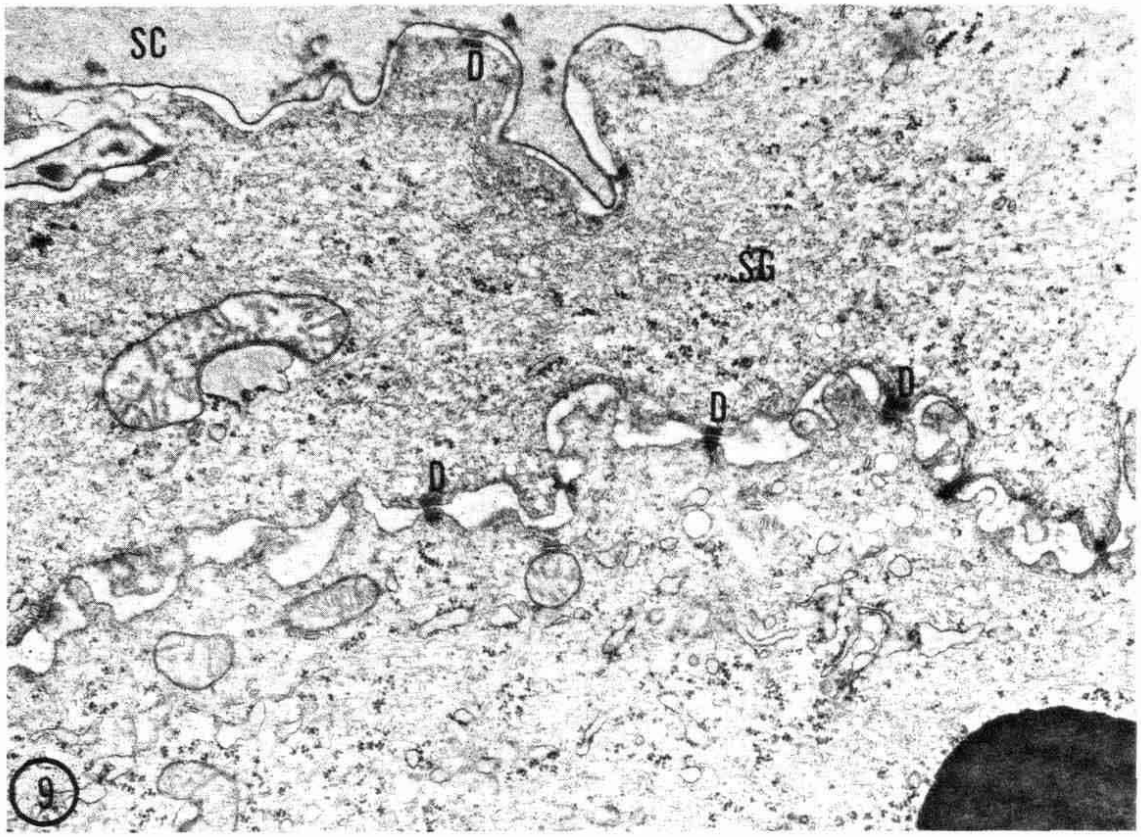
[a] Concentration of exfoliatin = 5 mg/ml media.

The results of these latter experiments suggest that: (1) the dermis is not responsible for the resistance of rat skin in vitro and (2) processing of exfoliatin via the dermis does not constitute a prerequisite for in vitro reactivity in mouse skin.

DISCUSSION

In Vitro and In Vivo Induction of Staphylococcal Scalded-Skin Syndrome in Various Species

An organ-culture system for the detection of exfoliatin reactivity was first described by McCallum [14], but her results are open to question



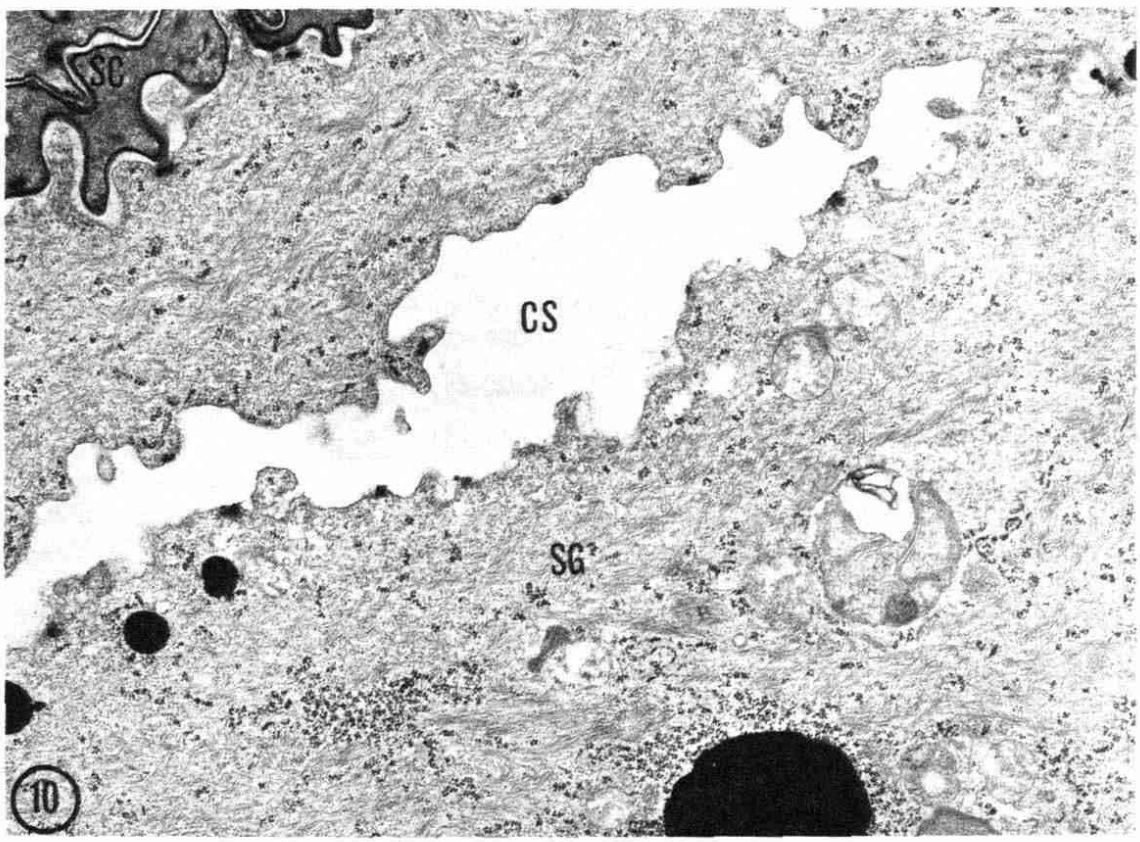
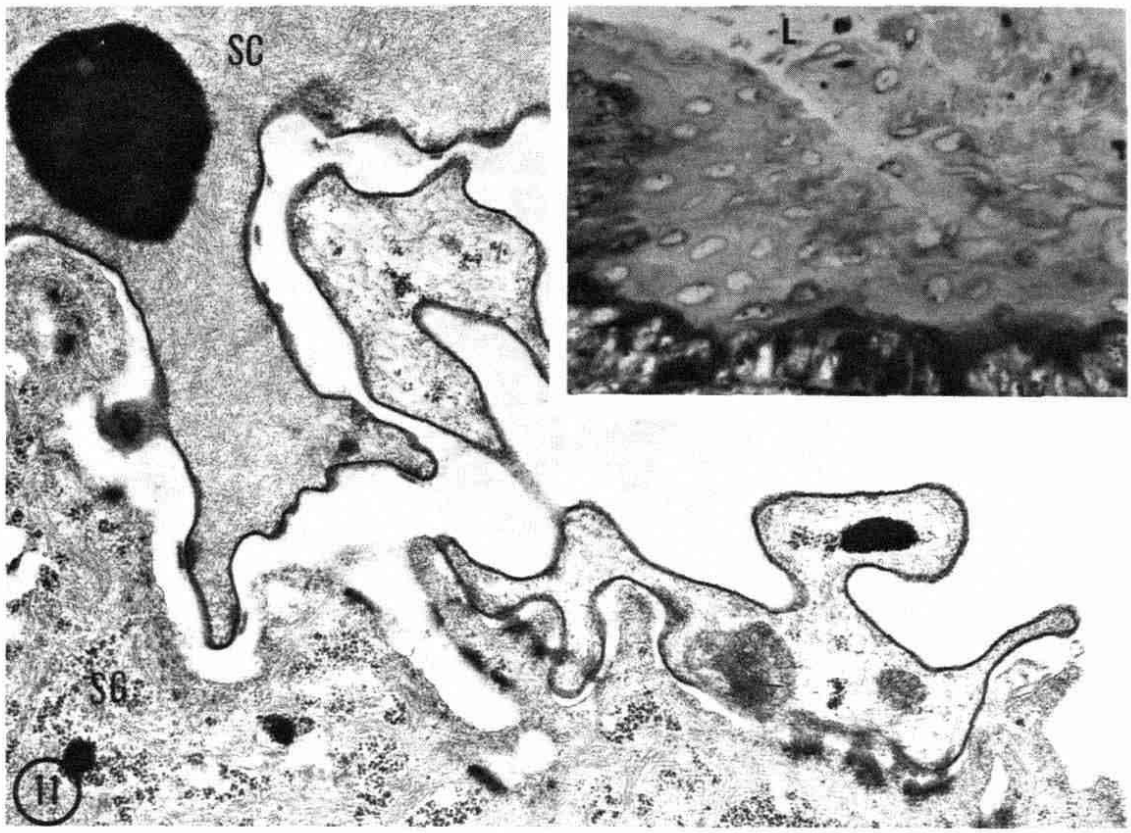
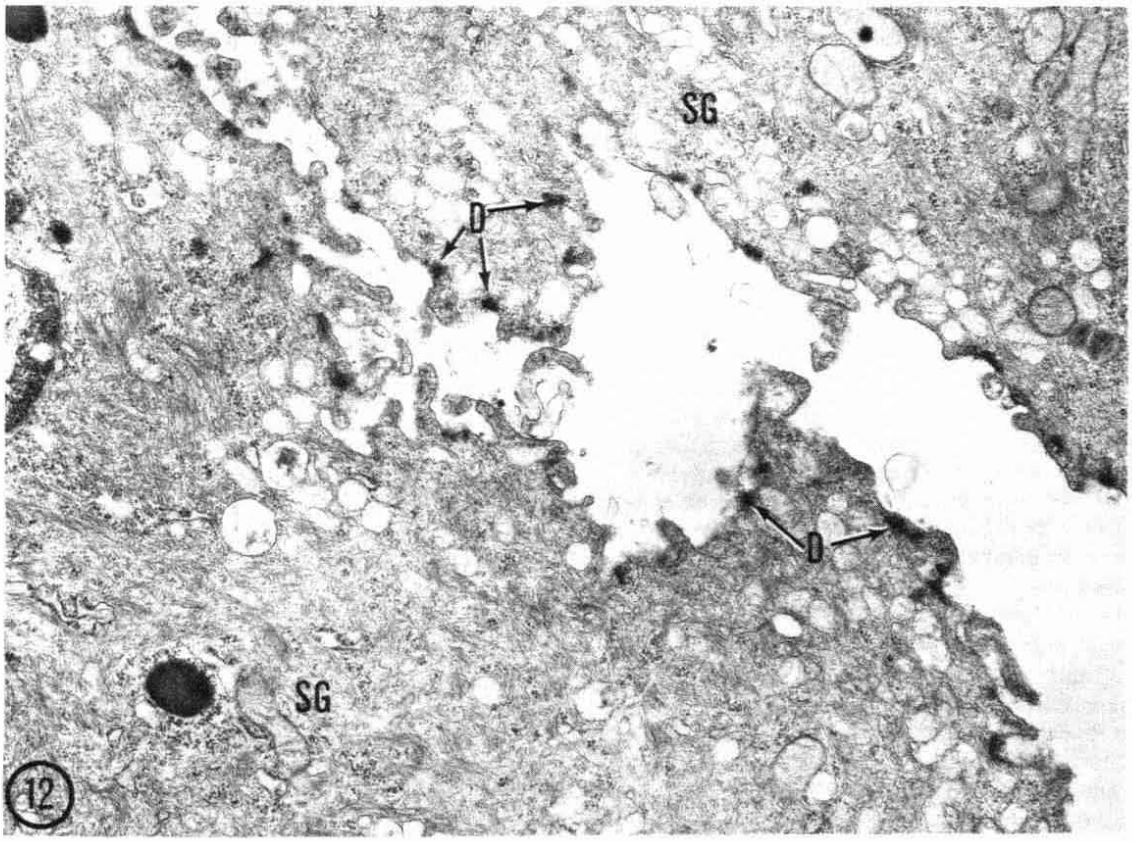


FIG. 9. Neonatal mouse esophagus, 2-hr control culture. The upper portion of the stratum granulosum (*SG*) and stratum corneum (*SC*) appear to be unperturbed. Intercellular domains contain amorphous, flocculent material punctuated by small desmosomes (*D*) (× 8,500).

FIG. 10–12. Neonatal mouse esophagus, exposed to exfoliatin for 2 hr in vitro. The cleavage plane (*CS*) is apparent in the mid-granular layer (*SG*) (*Fig. 10*), but may occur at the junction of the stratum granulosum and stratum corneum (*SC*) (*Fig. 11*), or in the deep granular layer (*Fig. 12*). Small desmosomes (*D*) and interdesmosomal regions seemingly separate simultaneously (*Figs. 11, 12*). *Fig. 11* (*inset*): Light microscopically the only evident change is enenhanced desquamation into the esophageal lumen (*L*). (*Fig. 10* × 4,500; *Fig. 11* × 27,500; *Fig. 11* (*inset*) × 1,500; *Fig. 12* × 15,000)


SC
L
SG
11


SG
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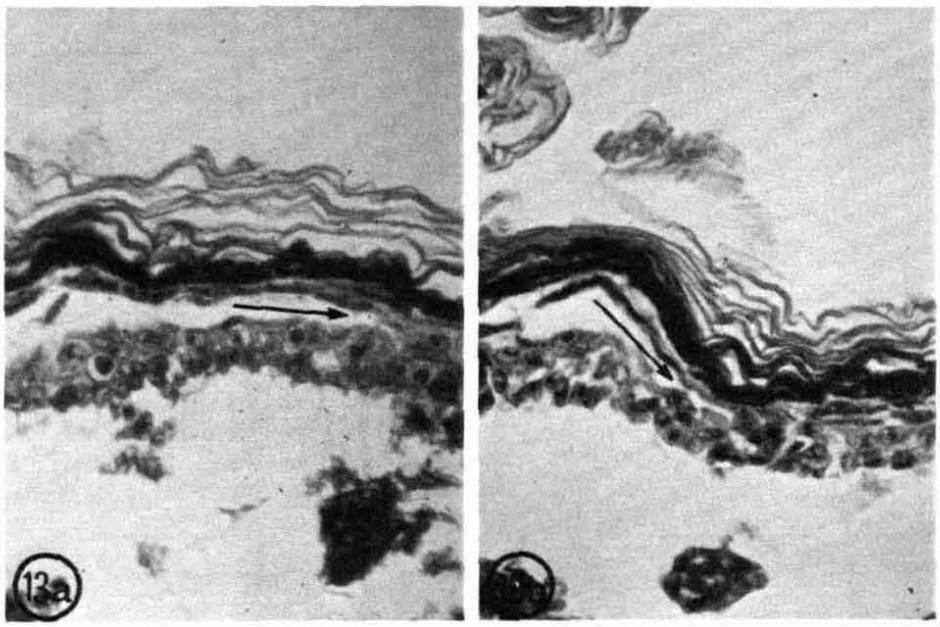

FIG. 13. Neonatal mouse epidermal sheets produced by EDTA incubation, then exposed to exfoliatin for 2 hr. Extensive intraepidermal division takes place most dramatically beneath the stratum granulosum (*arrows*) (*a*, *b* × 1,000).

because she assessed cleavage at a time (16 hr) when control specimens have often deteriorated sufficiently to manifest intraepidermal cleavage [11]. By using high enough concentrations of exfoliatin, we could achieve in vitro splitting more quickly (between 1 and 6 hr)—long before the onset of degenerative changes in control cultures [11].

In two preceding studies [11,19] utilizing the in vitro system, we demonstrated that hairy and glabrous sites in the same individual react the same in vitro. In the present study, we obtained dose–response curves reflecting the responses of individuals of diverse age and quantitatively compared the susceptibility of human and murine skin (Tab. III). As in our earlier investigations, differences in sensitivity were minimal. Two pitfalls in this type of experimentation may confound the unwary investigator and make comparisons difficult: (1) If the skin is not adequately scraped clean of subcutaneous tissue, diffusion of the toxin into the epidermis is greatly impeded or even abolished. (2) Intrinsic differences in the dermal thickness may result in misleading data. And either factor may prevent cleavage, even in potentially sensitive epithelia.

Here, we have shown that sensitivity to exfoliatin is not restricted to mice and man. Additional mammalian species examined proved to be sensitive, and the detection of other responsive species might well be anticipated if similar studies were conducted in broader scope. Moreover, we confirmed Kapral and Miller's findings that rats, rabbits, and chickens are not exfoliatin responsive [8], and added guinea pig, dog, and frog to the list of resistant species. Although hamsters seemed more refractory to exfoliatin than other sensitive species, the basic pattern of the syndrome was identical to that of the rest of the species tested, namely, a linear dose-response and the development of generalized toxic epidermal necrolysis in newborns with a contrastingly localized response in adult animals. Despite the limitation of our opportunity to test monkeys (for technical reasons), the data we obtained were wholly consistent with those compiled from studies of other sensitive species, and we may reasonably assume that the same pattern of disease occurs in monkeys as in hamsters and humans. Sensitivity is apparently an "all or none" phenomenon: responsive species react under all circumstances, and, conversely, resistant species remain absolutely resistant. Moreover, sensitivity to exfoliatin is a species-specific characteristic, possibly limited to mammals. Yet a close phylogenetic relationship (such as exists between mice and rats) does not necessarily imply a similar reaction to exfoliatin. Presumably, both sensitivity and resistance are inherent genetic properties of the epidermis and can perhaps be slightly modified by nonepidermal influences, e.g., the degree of dermal permeability, but they do represent the determining factor throughout the entire life span.

Extracutaneous Susceptibility to Exfoliatin

On the basis of our present observations, the action of staphylococcal exfoliatin is evidently quite tissue specific. Nonkeratinizing epithelia were uniformly unresponsive, and many keratinizing epithelia did not exfoliate. In human genital mucosa, monkey oral mucosa, and neonatal mouse esophagus and palate, enhanced sloughing occurred in apical regions. But the usual intraepithelial cleavage plane, considered typical of the classical staphylococcal scalded-skin syndrome [4,20], was lacking. The seemingly "intermediate"

response of mucosal epithelia could be attributable to factors such as the increased "turnover time" of these epithelia, to a paucity of receptors, or to the absence of a cohesive stratum corneum. These findings nonetheless correlate well with the clinical spectrum of the scalded-skin syndrome, i.e., patients do not manifest profound mucosal involvement [2,6].

All epithelia studied, keratinized or not, contained numerous desmosomes; yet in resistant tissues, none showed signs of perturbation, which may indicate that these organelles are not the primary target of exfoliatin, as initially proposed by Lillibridge et al [21]. Two plausible explanations for this resistance, based upon currently available information, warrant mention: First, Borysenko and Revel [22] recently observed that trypsin cleaves desmosomes *only* in keratinizing epithelia, while, conversely, EDTA cleaves desmosomes in nonkeratinized but not in keratinized epithelia. We may thus infer that the chemical composition of extracellular regions in various epithelia differs significantly. Accordingly, diversities in tissue susceptibility to exfoliatin could be traceable to either the presence or the absence of chemical substrates in the extracellular spaces. And there is the second possibility that tissue-specific, cell-surface exfoliatin receptors mediate responsiveness in epithelia of sensitive species. We favor this latter theory, since it is unlikely that the chemical composition of the intercellular spaces of resistant and susceptible species would differ significantly enough to explain the dramatic variations in response which we observed.

Species Resistance to Exfoliatin

We examined a number of credible explanations for the resistance of some species (and the susceptibility of others) to exfoliatin. First, we considered the feasibility that toxin-resistant skin actively elaborates a neutralizing or inhibiting substance. But we could find no evidence for the secretion of such a product by viable rat skin into culture supernatants (although it is still possible, though improbable, that other resistant species may secrete such substances). Next, we sought circulating plasma factor(s) which might inhibit exfoliatin, and again found that the rat possesses no circulating antiexfoliatin. And ultimately, we attempted to discover evidence of dermal interference with exfoliatin activity. Neither susceptibility in the mouse nor resistance in the rat, we learned, requires the presence of dermal elements. Stated otherwise, the presence of dermis does not serve to protect rat epidermis from exfoliating: the epidermis *itself* is refractory. It would seem most logical that the exfoliatin attacks a relatively specific keratinocyte receptor on the cellular surface or an intercellular substrate in susceptible species which is absent in resistant epithelia.

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